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DOI:
10.2217/fmb-2017-0130

Document Version
Peer reviewed version

Citation for published version (Harvard):
Hall, R 2017, 'Adapting to change: Interactions of Candida albicans with its environment', *Future Microbiology*, vol. 12, no. 11. https://doi.org/10.2217/fmb-2017-0130

Adapting to change: Interactions of *Candida albicans* with its environment

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Introduction

Candida albicans is a commensal of the oral, gastrointestinal and genital tracts of 80% of the population. However, during periods of immune suppression *C. albicans* is able to proliferate and damage the host. Infections range from chronic mucosal infections to life threatening systemic disease. *C. albicans* is estimated to cause 75 million cases of genital thrush each year, with 5-15% of women developing recurrent infection [1], and 400,000 deaths each year due to disseminated disease [2]. During commensalism the fungus is exposed to a plethora of biotic and abiotic environmental signals to which is must respond and adapt to, with many of these environmental cues activating the expression of virulence factors.

Morphogenesis

C. albicans is a polymorphic fungus. Originally *C. albicans* was thought to grow in four distinct morphologies (yeast, pseudohyphae, true hyphae and chlamydospores). However, our increased understanding of the pathobiology of *C. albicans* has identified several yeast-like morphologies of *C. albicans*, all of which play important roles in colonisation and pathogenesis of *C. albicans*, and are regulated by host environmental factors.

Yeast to hyphal transition

The ability of *C. albicans* to switch between yeast and hyphal forms is essential for pathogenesis, with strains locked in either morphology displaying significantly reduced virulence. The yeast to hyphal switch is regulated by many biotic and abiotic factors including elevations in temperature, CO_2, and pH, serum, hypoxia, starvation, and N-acetylglucosamine. Multiple transcription factors are required for morphogenesis, suggesting that a highly interwoven signalling network regulates the yeast to hyphal transition. From all the transcription factors identified to control morphogenesis, only constitutive expression of *UME6* induces hyphal growth [3],

suggesting that Ume6 is sufficient to drive morphogenesis. Basal levels of cAMP were thought to be essential for morphogenesis, but recent discoveries of hyphal induction in cAMP signalling deficient mutants confirm that only CO_2 induction of morphogenesis is truly dependent on cAMP [4].

Microbial signalling molecules inhibit hyphal growth, restricting *C. albicans* to yeast growth. Most recently *Enterococcus faecalis* has been shown to inhibit *C. albicans* hyphal formation through the bacteriocin EntV [5]. *C. albicans* can also control its own morphology through the secretion of quorum sensing molecules (QSMs). So far, farnesol and farnesoic acid have been shown to inhibit hyphal formation. Farnesol inhibits morphogenesis by targeting the cAMP-PKA pathway through direct inhibit inhibition of Cyr1 activity [6], while farnesoic acid inhibits morphogenesis through a novel pathway requiring Pho81 and Hot1 [7]. Bacterial QSMs also inhibit morphogenesis of *C. albicans* through modulation of the cAMP-PKA pathway [6, 8], making the cAMP-PKA pathway key in regulating the yeast-to-hyphal transition.

White to opaque switching

Yeast phenotypic switching is essential for mating in *C. albicans*, with opaque cells displaying higher mating frequencies than white cells. The white-opaque switch is regulated via the Wor1 master regulator, although other regulators have been identified [9]. Due to the involvement of several transcription factors in the regulation of white-opaque switching, and the extended cross-talk between environmental sensing pathways, the external environment can affect switching frequency. Until recently, mating was thought to require homozygosis of the mating type locus (MTL). However, white-opaque switching has been observed in a/α cells, all be it at lower frequencies, in response to elevated concentrations of CO_2, [10]. This low frequency switching in response to host environmental cues increases the possibility that sexual mating occurs in the host. However, this switching may also serve other important functions during commensalism. For example, opaque cells are better colonisers of skin, have an altered cell wall composition, and are not recognised by neutrophils [11]. These key characteristics of opaque cells make then an ideal cell type for commensalism.

Grey and GUT cells

The most recently identified yeast-like morphologies of *C. albicans* are the grey and gastrointestinally induced in transition (GUT) cells. The grey cells are an intermediate, but distinct cell type between the white and opaque cells making the mating switch a tripartite switch. The function of the grey cells remains to be determined, but this morphology appears to have increased growth rates at mucosal sites, including the oral mucosa, suggesting that the grey morphology may provide a selective advantage in this niche [12]. Like grey and opaque cells, GUT cells are elongated yeast cells. However, GUT cells appear specialised for colonisation of the gastrointestinal tract, and display enhanced colonisation of this niche over the other yeast like morphologies [12].

Environmentally induced Cell wall remodelling

The fungal cell wall is a dynamic organelle that surrounds the exterior of the fungus. As a consequence the cell wall is the initial point of contact between the host and the fungus, and many of the cell wall components act as pathogen associated molecular patterns (PAMPs) that are recognised by the innate immune system [13]. For some time it has been appreciated that the change in morphology from yeast to hyphal growth requires significant cell wall remodelling. However, more recently the ultrastructure of the cell wall has been shown to be responsive to the environment, independent of cell morphology [14]. This active cell wall remodelling has important immunological implications for infection and may, in part, explain why the immune response to *C. albicans* varies depending on the infection niche.

Environmental modulation of mannan

The outer layer of the *C. albicans* cell wall is comprised of fibrillar mannoproteins. These cell wall proteins are highly glycosylated with short linear *O*-linked mannan and complex branched *N*-linked mannan. Growth in different environmental conditions has been shown to influence the structural complicity of the *N*-linked mannan [14]. For example, growth at extremely low pH (pH2) results in decreased incorporation of phosphomannan via chemical cleavage of the phosphodiester bond

and reduced incorporation of β-mannose units into the acid stable *N*-linked mannan, promoting terminal α-1,3-mannose [14]. Growth on blood agar also reduces mannan complexity resulting in reduced side chain and terminal β-1,2 mannose units [14]. Although differences in *N*-mannan structure and composition in response to different environmental conditions are well documented, the mechanisms that mediate these changes are not. According to global transcriptional studies, many of the genes involved in mannan biosynthesis are differentially regulated in response to environmental cues, but the molecular mechanisms mediating these changes have not been investigated. Given that this outer mannan shield protects the immunostimulatory β-glucan, understanding the regulation of mannan biosynthesis is an important understudied aspect of *Candida* biology.

Environmental modulation of glucan exposure

The inner structural layer of the cell wall contains β-1,6 and β-1,3-glucan. The former glucan structure is readily recognised by the innate immune system via the C-type lectin-like receptor Dectin-1 [15]. However, recently *C. albicans* has been shown to actively regulate the amount of β-glucan that is exposed to the immune system enabling *C. albicans* to either evade or stimulate the innate immune system in response to specific environmental cues. As infection progresses glucan becomes increasingly exposed on the surface on *C. albicans* initiating strong proinflammatory immune responses [16]. Sub-MIC concentrations of the antifungal drug caspofungin also promote β-glucan exposure, activating the immune system to aid fungal clearance [17].

In the vaginal niche the main carbon source is lactate, which is produced by lactobacilli. Lactate has profound effects on the cell wall of *C. albicans*. One of the most striking observations is that lactate induces concealment of β-glucan [18]. This masking of β-glucan is regulated via Crz1, but does not require other components of the well-established calcium-signalling pathway. Instead, lactate signals through the Gpr1 receptor; suggesting that lactate can either be used as a signalling molecule or for metabolism [18]. Therefore, *C. albicans* may use lactate sensing to discriminate between the oral and vaginal mucosa and conceal β-glucan to promote

commensalism within the female reproductive tract. However, the lactate produced by lactobacilli maintains the vaginal mucosa at pH4 to prevent bacterial vaginosis. A recent study confirmed that acidic environments promote exposure of β-glucan, even in the presence of lactic acid [19]. This enhanced β-glucan exposure was suggested to attribute to the clinical symptoms of vulvovaginal candidiasis (VVC), as acidic grown cells initiated a strong proinflammatory innate immune response, resulting in significant recruitment of neutrophils, similar to the immunopathology of VVC. The signalling mechanism that mediates the active remodelling of β-glucan in response to environmental pH is unresolved, but is not mediated via Rim101, Crz1 or Hog1 [19]. Given the importance of β-glucan exposure in innate immune recognition it is possible that *C. albicans* has evolved several mechanisms to control β-glucan exposure to promote its success as both a commensal and pathogen.

Environmental modulation of chitin

Chitin forms a minor (3-5% by dry weight), but essential component of the cell wall. Upon encountering stress, *C. albicans* up regulates chitin biosynthesis to reinforce the cell wall and provide protection from the surrounding environment. This enrichment of chitin is regulated by the cell wall salvage, HOG1 and the calcium/calcinurine pathways [20]. As demonstrated for β-glucan, both caspofungin and low pH induce chitin synthesis and PAMP exposure [19], which suppresses the immune responses. Whether β-glucan and chitin exposure must occur concurrently is unknown, but non-concurrent exposure would enable *C. albicans* to manipulate host immune responses to its own advantage.

Conclusion

The interaction of *C. albicans* with its environment governs multiple biological processes including cell morphology and cell wall biogenesis. This plasticity of *C. albicans* enables the fungus to easily switch between commensal and pathogenic growth. Understanding the complexity of these interactions is required to fully appreciate the host-*C. albicans* interaction.

Acknowledgements

RAH is supported by an MRC Career Development Award (MR/L00903X/1).

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